Specific Aims

Diffusion MRI (dMRI) is a powerful, non-invasive tool for characterizing neurological tissue microstructure on a macroscopic scale, and is widely used in both research and clinical settings. New methods of reconstructing orientation distribution functions (ODFs) from dMRI data are rapidly being developed, each seeking to identify and model the distribution of distinct, sub-voxel axon fiber populations. The 3D orientations of these fiber populations are passed into tractography algorithms, which are used in connectomics studies to estimate the paths of individual nerve tracts throughout the brain.

Efforts to validate both ODF reconstruction methods and tractography results have typically relied on serial optical histology. For ODF validation, a computer vision technique called "structure tensor analysis" uses image intensity gradients to estimate voxel-wise fiber orientations in the high-resolution histology data. These orientation estimates are then binned across regions of interest the size of a dMRI voxel in order to form ground-truth ODFs, which are compared pair-wise to ODFs reconstructed from dMRI data of the same specimen. Tractography validation is generally performed using neural tracers injected into a small number of seed sites before the sample is sacrificed. Voxel-wise comparisons between dMRI tractography results and the ground truth tracts identified in histology from these tracers are then used to evaluate the performance of different tractography algorithms.

Both of these validation efforts rely on the labor-intensive process of physically sectioning, staining, and optically scanning hundreds of slices of the tissue of interest. The slices are necessarily at least 10-20 times thicker than the achievable in-plane resolution (~5000 nm vs. 250 nm), yielding non-isotropic volumetric reconstructions; distortions introduced by sectioning further limit the ability to align the slices and extract faithful information on the 3D orientation of fiber populations.

We are pioneering the use of synchrotron micro computed tomography (microCT) as a means of performing isotropic, 3D imaging of whole mouse brain specimens at sub-micron resolution, with the potential ability to resolve every axon in the brain. We propose to develop and optimize a pipeline to use microCT data to validate and characterize dMRI ODF reconstruction and tractography algorithms. The results of this work will address the limitations of previous histology-based studies, while generating a publicly available whole-brain tractography atlas through a combination of white matter image segmentation and "ground-truth" tractograms generated from microCT ODFs. The specific aims are:

Aim 1: Optimize microCT data acquisition to maximize axon fiber bundle contrast. Specimens are stained with uranyl acetate, osmium tetroxide and lead citrate prior to microCT imaging. The concentrations and staining protocol for these agents will be optimized along with the synchrotron beam energy to maximize the contrast of axon fiber bundles in the microCT data. Additionally, we will install and characterize a new detector system with a higher resolution and wider field of view to simplify and improve the microCT image reconstruction, which currently relies on a mosaic data-stitching method to image whole mouse brains.

Aim 2: Validate dMRI reconstruction methods using ground-truth microCT ODFs. We will compute ground-truth ODFs from the microCT data across a whole mouse brain using structure tensor analysis. The microCT ODFs will be compared to ODFs calculated using a variety of reconstruction methods on dMRI data from the same specimen, generating algorithm-specific spatial maps of dMRI accuracy. We will investigate patterns in the morphological features of failure regions in an effort to better understand modeling errors in the dMRI reconstruction methods.

Aim 3: Generate a ground-truth tractography atlas from microCT ODFs and segmented axon fiber bundles. We will generate a 3D tractography atlas by running dMRI tractography algorithms on the ground-truth ODFs from microCT. This will yield the "best-performance" results of these algorithms; having mitigated the effects of inaccurate ODFs from dMRI, this approach will allow us to understand the inherent limitations in the problem of estimating connective tracts from orientation data. Tractogram results will also be validated against segmented axon fiber bundles from the microCT intensity data for algorithm-specific characterization.

Upon completion, this project will generate a comprehensive validation map for both dMRI reconstruction methods and tractography, exploiting the advantages of a novel dataset uniquely tailored to this purpose. The ground-truth tractography atlas will be made publically available as an unprecedentedly comprehensive tool for studies into the future role of dMRI in connectomics research.